

Strategies to Enhance Effects of Cellular Immunotherapy

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Lasker Clinical Scholar

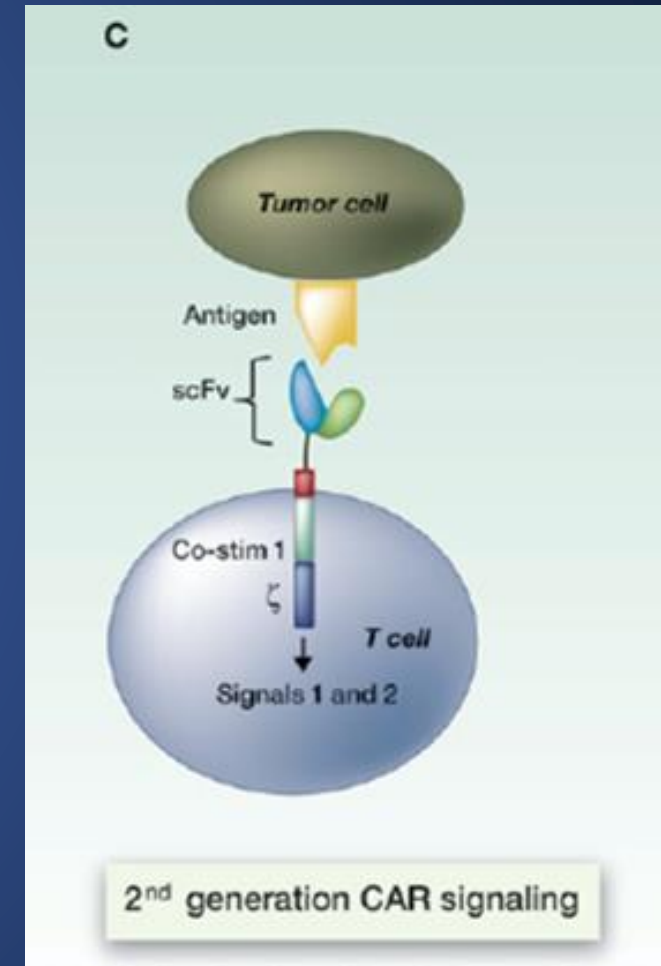
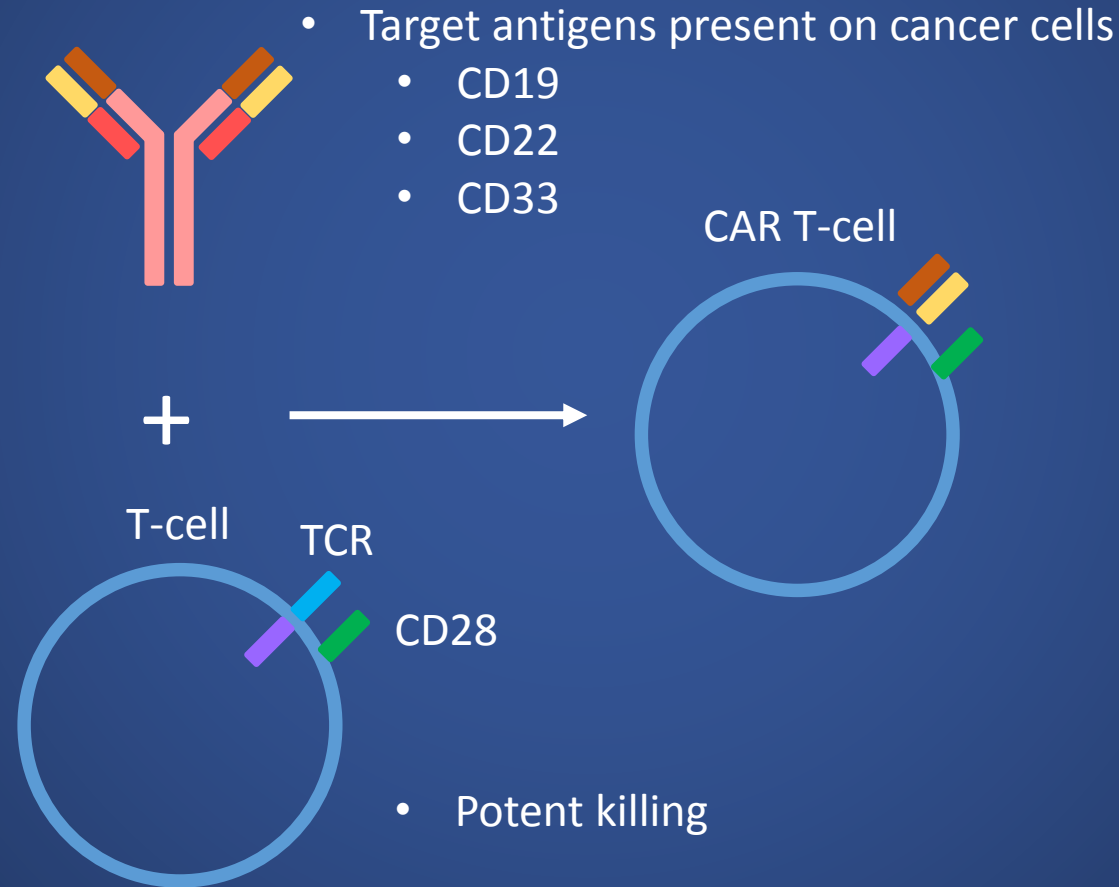
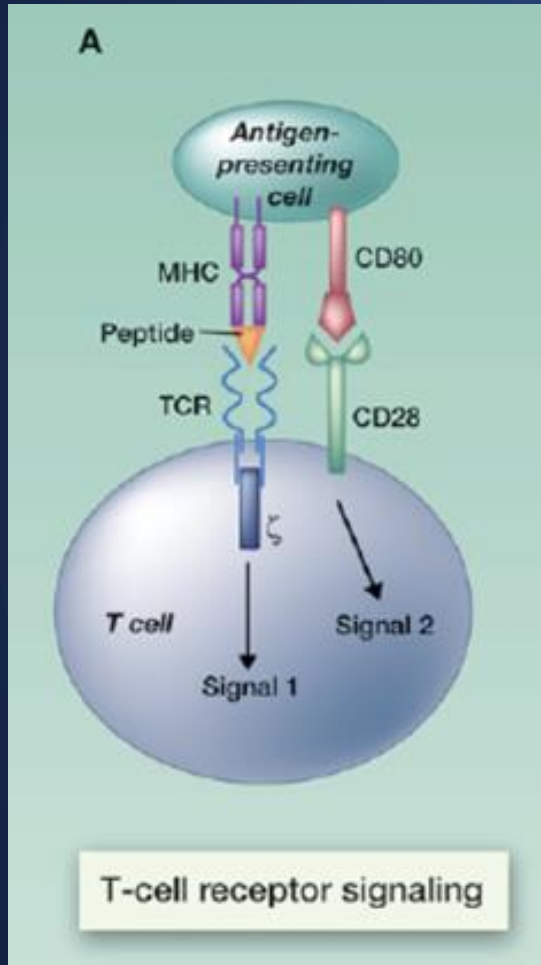
Pediatric Oncology Branch

National Cancer Institute

Myelodysplastic Syndromes Symposium

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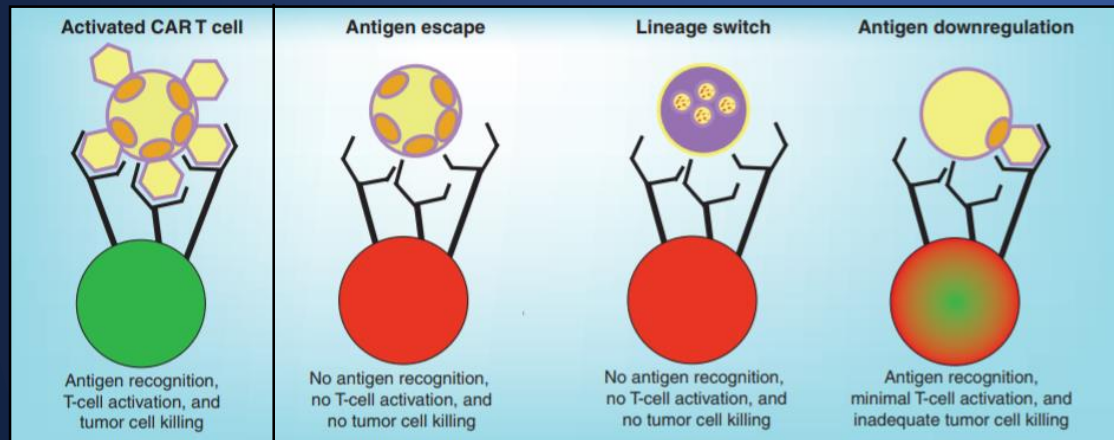
What is Chimeric antigen receptor (CAR) T cell therapy?



How effective is CD19 CAR T-cell therapy in refractory pediatric B-ALL?

- 60-90% initial remission rate¹⁻⁴
- 29-45% long-term relapse rate⁵

General mechanisms of disease resistance:



Adapted from (5)

Table 1. A summary of antigen escape in CD19 CAR trials for ALL

Trial	Relapse rate	CD19-negative relapse rate
Children's Hospital of Philadelphia phase I	36% (20/55)	24% (13/55)
Novartis phase II (ELIANA)	33% (20/61)	25% (15/61)
Seattle Children's Research Institute phase I	45% (18/40)	18% (7/40)
NCI phase I	29% (8/28)	18% (5/28)
Memorial Sloan Kettering phase I	57% (25/44)	9% (4/44)
Fred Hutchinson Cancer Center phase I	31% (9/29)	7% (2/29)

Adapted from (5)

Mechanisms of resistance to CD19 CAR:

- CD19 genetic mutations and loss of heterozygosity⁶
- Splicing variants⁷
- Loss of CD81⁸
- Lineage switch⁹

1. Lee DW et. al, Lancet 2015; 2. Davila ML et. al., Sci Transl Med 2014; 3. Maude SL New Engl J Med 2014; 4. Kochenderfer JN J Clin Oncol 2014 ;
5. Majzner, RG and Mackall, CL Cancer Discov 2018
6. Orlando, et. Al. Nat. Med. (2018); 7. Sotillo, E. Et. Al. Cancer Discov (2015); 8. Braig F et. Al. Blood (2017) 9. Jacoby, E. Et. Al, Nat. Commun (2016)

Is there still a role for the cancer biologist in the era of CAR T cell therapy?

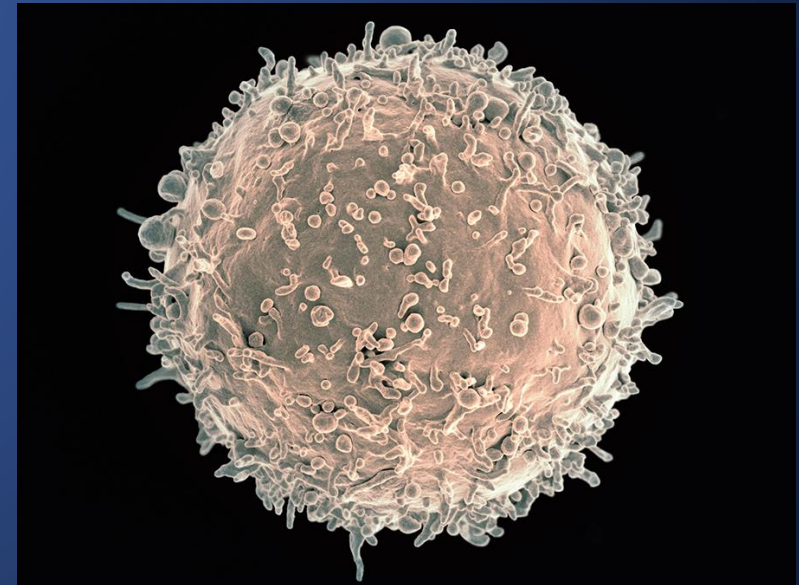
Can we change target expression to increase the efficacy of CAR-targeting therapies?

Small molecule screen for molecules that alter the antigens presentation on the cancer cell

Can we identify new antigenic targets?

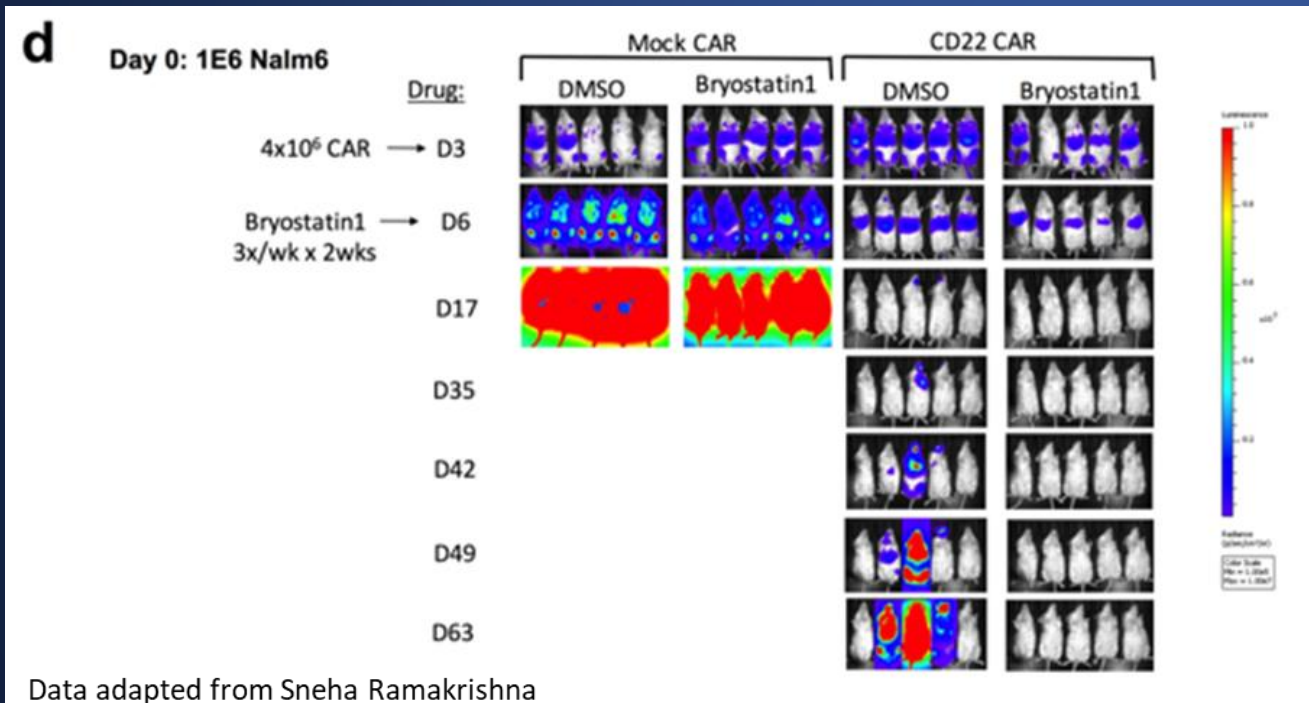
Antigen mapping and new antigen target discovery

- Surfaceome MS + high coverage total RNAseq
(Collaboration with Richard Aplenc – CHOP)



Can we change target expression to increase the efficacy of CAR-targeting therapies?

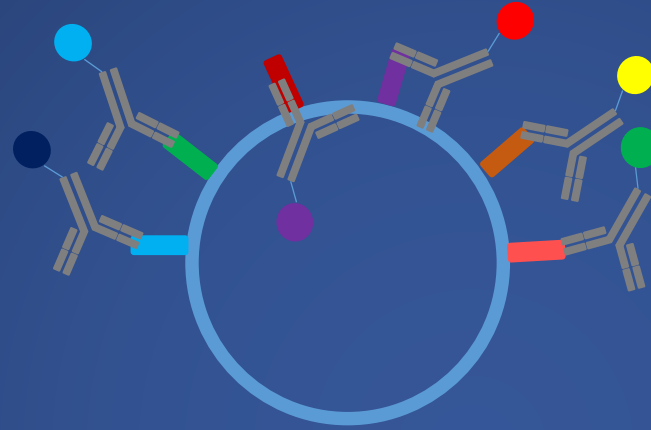
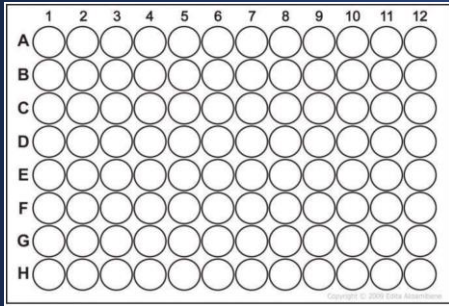
Increasing target expression with bryostatin1 improves CD22 CAR function:



Project goals:

- Primary goal: Identify drugs that increase CD19 surface expression
- Additional goals:
 1. Identify drugs that affect other CAR targets (CD22, TSLPR, CD33, FLT3)
 2. Generate mechanistic data on CD19 regulation
 3. Analyze the clinical relevance of drugs that change CAR target expression

Design of a screen for drugs that affect antigen expression



- 96 well format with tiled drugs
- Leukemia cell line

- Screen with flow cytometry
- Markers
 - CD19
 - CD22
 - CD33
 - FLT3
 - TSLPR
 - CD81
 - Viability

CAR targets

Associated with CD19

Drugs that increase antigen expression

- Mechanism
- *In vitro* and *in vivo* CAR-T efficacy

CD19

1.169403	1.113732	1.123277	1.169884	1.156105	1.108182	1.093348	1.093022	1.049999	1.068598	1.103324	0.908903
1.038466	1.113154	0.922068	0.994501	0.840922	1.03842	1.027816	1.009682	0.964807	1.013217	0.1358798	1.010342
1	0.995648	1.032208	1.000538	0.994815	0.990462	0.952287	0.934225	0.94114	0.576763	0.94406	1.036576
0.992509	1.022656	0.923362	0.980863	0.997887	0.958462	0.992469	0.930361	0.904473	1.011599	0.984902	0.846792
1.078064	0.999812	0.920232	0.904065	0.939019	0.835769	0.953597	0.985812	0.85453	0.956335	0.950083	0.769049
1.015026	0.961825	0.95301	1.064547	1.012475	1.008018	0.971353	0.891477	0.949625	0.979334	0.864508	0.782588
0.931209	0.94013	0.964245	0.9267	0.885644	0.876512	0.929663	0.928629	0.838311	0.900722	0.874136	0.67389
0.9116	0.926964	0.919717	0.925298	0.93699	0.982307	0.92299	0.96078	0.956245	0.894345	0.879785	0.130956

CD22

1.057258	1.022801	1.018096	0.96114	0.836988	0.926704	1.478024	0.873999	0.707406	0.878392	0.873938	0.754975
0.802367	1.284063	1.105573	0.652288	0.661047	0.826298	0.946021	0.531488	0.584083	0.800692	0.407106	0.914129
1	1.029426	1.082618	1.042876	1.039566	1.024207	1.016348	1.168858	1.195156	0.933564	1.038754	1.076817
1.120372	1.127484	1.060167	0.988078	1.092321	1.111527	1.05297	1.073284	1.031538	1.133293	1.08851	1.250228
1.188753	1.128186	0.992692	0.971713	1.038136	0.840713	1.049726	0.988526	0.997711	1.05086	1.051302	0.858011
1.242713	1.178185	1.190279	1.214197	1.199151	1.231994	1.172489	1.062791	0.950719	0.762021	1.047603	0.853425
1.071244	1.098556	1.120165	1.081677	1.049397	1.048783	1.240441	1.11242	1.027365	1.139792	1.048443	0.777163
1.146644	1.141068	1.043999	1.083648	1.12508	1.705055	1.044459	1.239742	1.22621	1.053216	1.019152	1.121822

CD81

1.529106	1.439147	1.484833	1.403049	1.032322	1.381128	0.90533	1.308293	1.491145	1.292698	1.454417	1.82319
1.252016	1.189177	0.965509	1.13737	1.075873	1.221294	1.197866	1.446996	1.158432	1.243563	0.426843	1.23803
1	0.965573	1.10958	1.043154	1.113164	1.073822	1.058854	1.014614	1.008815	0.679657	0.944328	0.983331
0.997865	1.055434	0.790292	1.124715	0.910916	0.861714	0.835936	0.942215	2.23197	1.19445	0.974484	0.664425
1.312257	0.900908	0.789374	0.740731	0.830557	0.696074	0.83095	0.815879	0.808484	0.862321	0.84467	0.72922
0.968115	0.963201	0.755901	0.905834	0.892966	0.686382	0.788509	0.76669	0.618685	0.924443	0.849881	0.71355
0.705675	0.693569	0.744746	0.682186	0.732785	0.688119	0.723041	0.68789	0.706142	0.784737	0.736952	0.606124
0.600507	0.602132	0.634598	0.585141	0.611337	0.469693	0.701871	0.610003	0.6136	0.640803	0.622864	0.614511

CD33

1.128069	1.057382	1.017255	1.163594	0.862587	0.964335	0.715848	0.583519	0.709988	0.710411	0.575745	0.798892
0.359978	0.61786	1.072983	0.243471	0.739305	0.374815	0.8	0.216296	0.495556	0.226667	0.642087	0.881132
1	1.185186	1.217765	1.153156	1.043767	1.170749	1.153128	0.2051852	1.948148	3.96296	1.831481	1.896296
1.551962	1.506814	1.385569	1.46931	1.403092	1.427379	1.422889	1.387283	1.50416	1.837898	1.647788	1.44849
2.182248	1.615789	1.365307	1.094663	1.194354	0.762092	1.19618	1.272143	1.408935	1.662846	1.77467	1.214096
1.825513	1.514024	1.650587	1.404996	1.798675	1.855946	1.692736	1.288254	59.5877	1.214994	1.47951	1.54606
1.574438	1.553787	1.709281	1.550312	1.312287	1.399547	1.722851	1.626877	1.577125	2.074074	2.081481	2.340741
1.66289	1.762876	1.735953	1.868957	1.821675	2.013225	1.825669	2.057852	1.881489	1.68779	1.86333	1.883473

FLT3

1.20829	1.164975	1.178768	1.208349	0.971459	1.202899	0.920245	1.116929	1.055353	1.205031	1.249658	1.181046
1.108397	0.929486	0.537475	1.030315	1.041149	1.119674	1.091568	1.049864	0.884859	1.162285	0.568557	1.164283
1	0.995843	1.037455	1.090509	1.046882	1.06174	0.986121	0.973708	0.968268	0.715322	0.899665	0.977335
1.05309	1.049713	0.918896	0.881144	1.070913	1.021761	0.989377	0.932744	0.872557	1.054645	1.037673	0.700901
1.143599	1.065255	1.050653	0.939022	0.950829	0.866761	0.943609	0.819022	0.800422	0.930955	0.910949	0.806914
0.996285	0.851994	0.855221	1.10792	1.118726	1.033253	0.938354	0.821731	0.833029	0.839344	0.786823	
0.860252	0.832251	0.908769	0.839875	0.811777	0.843592	0.922742	0.853593	0.821873	0.816863	0.825023	0.656458
0.807771	0.918498	0.832537	0.870567	0.883455	0.852825	0.830275	0.994326	0.923128	0.864419	0.871123	0.899721

TSLPR

1.02354	1.033221	1.071969	1.027521	1.410666	1.050704	1.132287	1.083175	1.092279	1.166244	1.175378	1.197821
1.046272	1.095798	1.07988	1.05952	1.144255	1.092409	1.069307	1.118812	1.112211	1.465347	0.751432	1.140351
1	0.968598	0.98063	0.972355	0.990042	1.064514	1.020093	1.019802	1.036304	1.069307	1.25413	1.049505
0.968537	0.939529	0.96019	0.995147	1.019888	1.003364	1.001605	1.049294	0.993342	1.127269	1.082105	1.052194
0.993403	0.994319	1.093239	1.09137	1.077695	1.122342	1.001438	1.08908	0.996968	0.968083	1.014418	1.04534
0.937722	0.900407	0.910713	0.964542	0.972843	0.994293	0.919971	0.929113	0.9314881	1.685675	1.039331	1.01583
0.910884	0.936165	0.938553	0.915954	0.929647	0.950631	0.950111	0.967404	1.005607	0.914191	0.891089	1.102331
0.913701	0.981825	0.861233	0.901404	0.882471	0.988034	0.909704	0.969272	0.963431	0.953008	0.982723	0.693755

Viability

1.082718	1.088254	1.087368	1.021717	0.613743	0.98213	0.98233	1.027537	0.968814	1.010092	0.975748	0.67304
0.993361	1.008017	0.823312	0.899419	0.638333	1.029248	1.037604	1.006964	1.002786	0.436023	1.006964	0.890628
1	0.983044	0.972301	1.031764	0.987571	1.015612	1.029248	1.029248	1.029248	1.010961	1.015042	0.94354
0.934601	1.017687	0.936259	0.949605	0.948421	0.94162	1.003726	1.081004	1.100369	0.767203	0.948863	0.740899
0.922382	0.975196	0.881039	0.961936	0.944795	0.963413	1.001775	1.070699	1.001509	0.868009	0.939959	0.76488
1.036627	1.018541	1.012919	0.952782	0.957787	0.930404	0.960867	1.024185	1.111341	0.765386	0.953125	0.728246
0.974233	0.983334	0.946639	0.96982	1.007537	0.965838	0.985233	0.946419	0.675047	0.941504	0.976323	1.187273
0.849353	0.880366	0.890357	0.856206	0.870561	0.964908	0.89883	0.905116	0.923283	0.824694	0.78021	0.917521

24 hrs

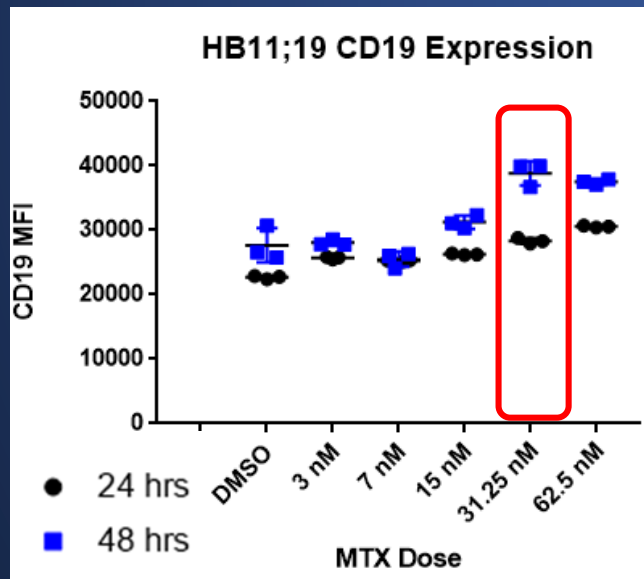
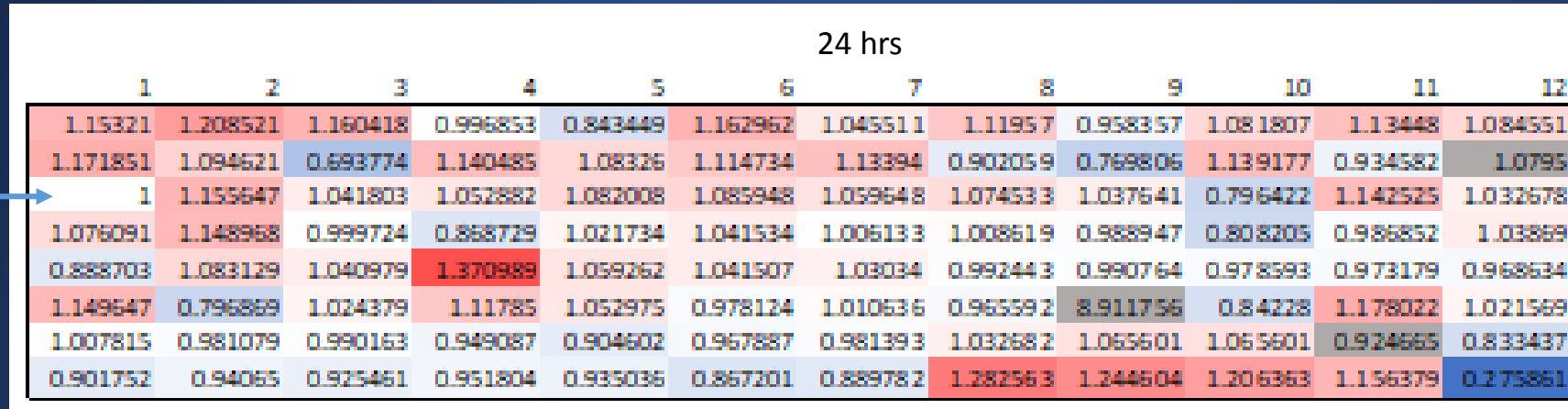
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1.171851	1.094621	0.693774	1.140485	1.08326	1.114734	1.13394	0.902059	0.769806	1.139177	0.934582	1.0795
1	1.155647	1.041803	1.052882	1.082008	1.085948	1.059648	1.074533	1.037641	0.796422	1.142525	1.032678
1.076091	1.148968	0.999724	0.868729	1.021734	1.041534	1.006133	1.008619	0.988947	0.808205	0.986852	1.03869
0.888703	1.083129	1.040979	1.730898	0.959624	1.041507	1.03094	0.944403	0.90764	0.978593	0.971319	0.688234
1.149647	0.796869	1.024379	1.11785	1.052975	0.978124	1.010636	0.965592	8.911756	0.84228	1.178022	1.021569
1.007815	0.981079	0.990163	0.949807	0.904602	0.967887	0.981399	1.032682	1.065601	1.065601	0.924665	0.833437
0.901752	0.94065	0.925461	0.951804	0.935036	0.867201	0.889782	1.282563	1.244604	1.206363	1.156379	0.275861

0.903418	1.027011	0.933315	0.453626	0.84638	0.904007	0.932852	0.911667	0.71874	0.908337	0.835109	0.812536
1.037791	0.958962	1.227698	0.947523	0.915463	0.985911	0.881118	0.50494	0.455407	0.912304	0.658291	0.794757
1	1.093283	0.946131	0.86899	0.896703	0.909461	0.840483	0.937375	0.87005	0.789876	0.846948	0.855461
1.135239	1.09589	0.843422	0.661442	0.906653	0.991365	0.911082	0.901367	0.849211	0.502527	0.809767	0.852335
0.625009	1.178956	1.025724	1.427301	1.004305	0.92401	0.817948	0.917754	0.81039	0.878829	0.841544	
1.181422	0.571445	0.958142	0.858936	0.84879	0.855125	0.984284	0.869914	0.744962	0.41175	1.512945	1.026709
1.053141	1.035625	0.969738	0.857274	0.764167	0.899923	1.085138	0.895607	0.844303	0.844303	0.797586	1.008088
0.973615	1.010142	0.911746	0.862634	0.814637	1.099073	0.785805	1.405344	1.320115	1.228427	1.362848	1.661049

1.462073	1.469894	1.393044	1.286648	0.906628	1.384709	0.882536	1.29836	1.41121	1.380348	1.27922	1.339172
1.198454	1.408665	0.81339	1.111609	1.135242	1.150872	1.134585	1.950197	1.320605	1.190881	0.908202	1.060273
1	1.106121	1.317784	1.006365	0.980604	1.028936	1.129408	0.975294	1.07623	0.732875	0.915109	0.950154
0.968998	0.905417	0.683705	1.131223	0.956715	0.978011	0.886204	0.92078	2.083162	1.251261	0.934916	0.758397
1.311612	0.960696	0.860403	0.864465	0.842877	0.987939	0.787707	0.844184	0.843356	0.791983	0.820083	0.698366
0.90663	1.199312	0.838325	0.949636	0.833184	0.76635	0.850925	0.793694	0.951936	1.267563	0.533783	0.83658
0.790521	0.722842	0.72802	0.688713	0.742518	0.762409	0.806043	0.752743	0.719403	0.719403	0.737111	0.683084
0.609249	0.598837	0.608772	0.612484	0.649777	0.546089	0.872722	0.607395	0.526826	0.55224	0.582055	0.564284

Results: Flow Heatmap for CD19 in REH cell line

DMSO



- MTX causes the greatest ↑ in CD19 fold change at 24 hrs in both lines
 - 1.37 fold change in REH, 1.49 in HB11;19
- Optimized dose and time (30 nM, 48 hrs)

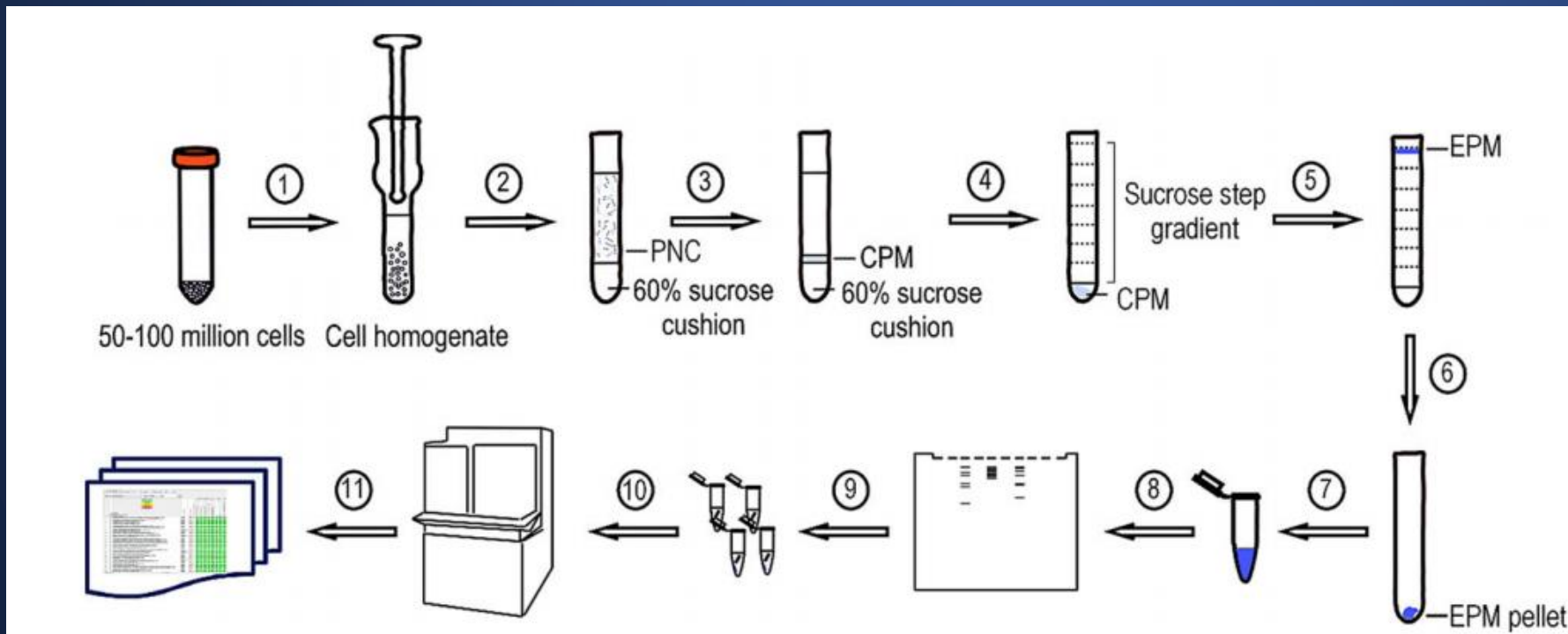
	Median (IQR) MFI Control	Median (IQR) MFI MTX 30 nM, 48 hrs	P value for Control vs. MTX
REH	6967 (5919, 7260)	10058 (8280, 13582)	P = 0.008
HB11;19	27993 (27305, 35425)	53448 (50772, 57764)	P = 0.016

MTX = methotrexate

MFI = geometric mean fluorescence intensity

Can we identify new antigenic targets?

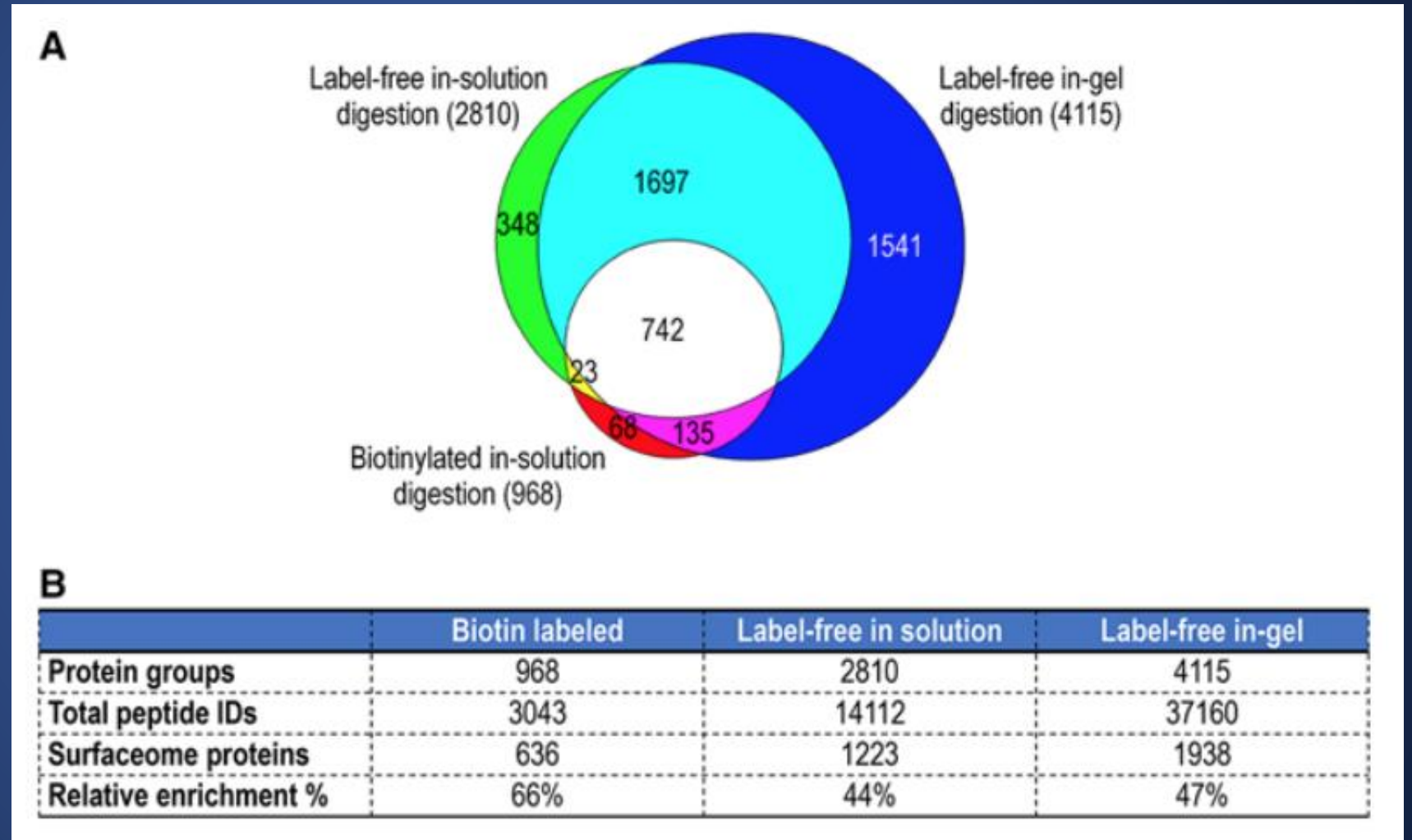
- Defining the proteins that compose the cellular membrane remains challenging
 - Low abundance
 - Hydrophobic/lipid characteristics
 - Low membrane to cytoplasm ratios
- Gene expression profiling has not allowed us to draw reliable conclusions about the events at the protein level given the poor correlation between mRNA and protein expression

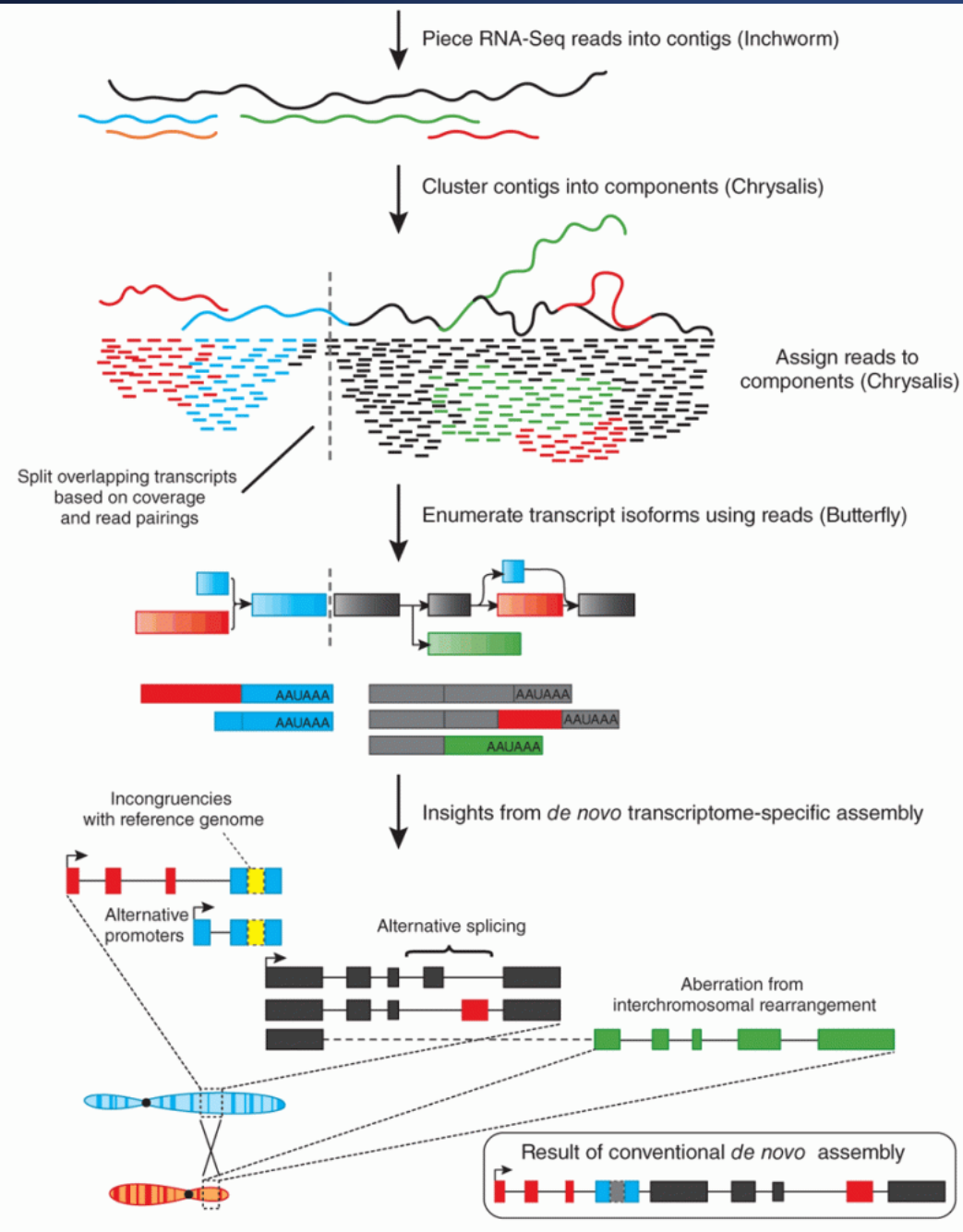


Development of a robust,
label-free, nonaffinity-
purified MS workflow

Can we identify new antigenic targets?

- Human surfaceome is predicted to contain around 3700 proteins based on in silico prediction
- Using standard procedures, no MS study has reported more than 870 PM associated proteins
- Identified 4115 proteins
- 1938 (47%) were previously annotated as plasma membrane



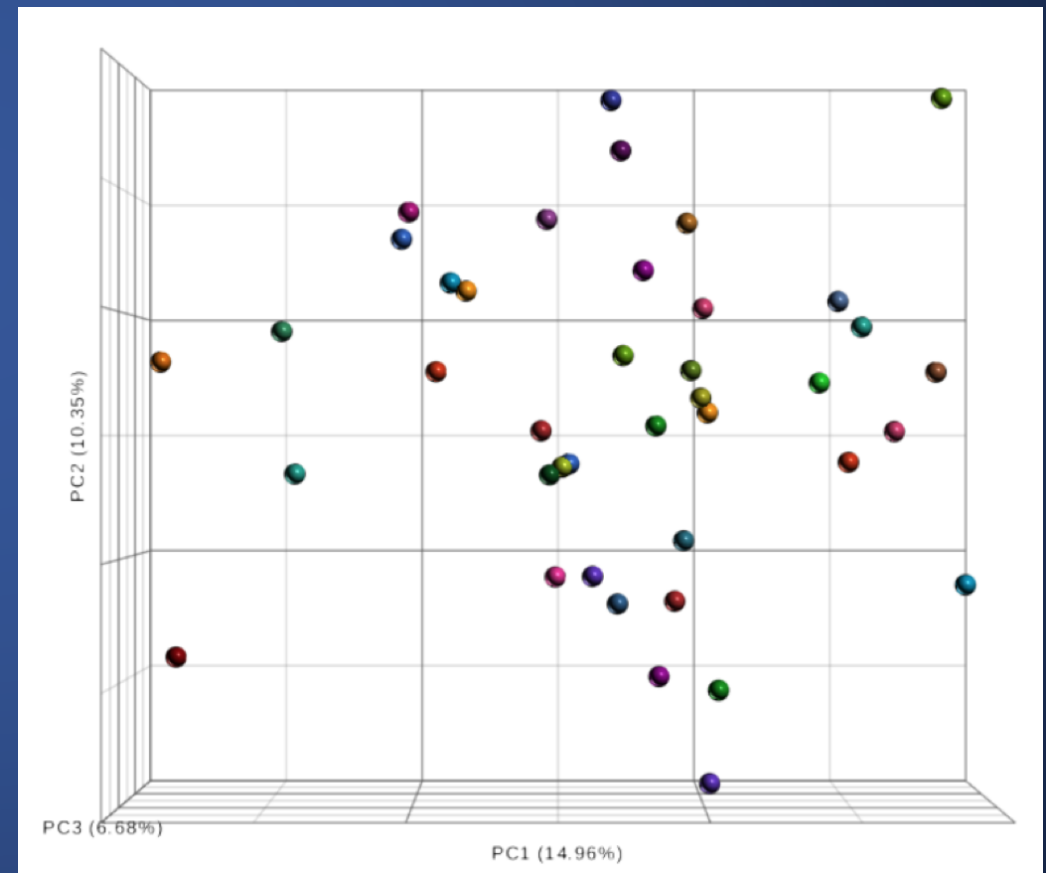


Goal: generate a sample specific “reference transcriptome”

Assembled reference transcriptome  Map MS data

- Map to known reference transcriptome - Ensembl
- De novo assembly of RNAseq data

High coverage ribosome depleted RNAseq of 6 AML cell lines
40 AML patient samples data mapped and de novo assembly completed



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- Tina Glisovic-Aplenc – Childrens Hospital of Philadelphia